Innovations

The Use of Transgenic Mice for Environmental Health Research

One of the most remarkable technical advances in biomedical research has been the development of transgenic mice. Transgenic mice can be made by using a transgene constructed with recombinant DNA techniques to randomly insert a new gene into the genome of the mouse or by mutating a targeted gene. A randomly inserted transgene usually adds to the genetic repertoire of the animal, whereas a targeted mutation usually produces a functional "knock-out" of a gene. Transgenic mice are a unique tool for understanding how interactions between individual genes and the environment affect human health.

Randomly Inserted Transgenes

Most transgenic mice are made by injecting a transgene into fertilized eggs. The eggs are removed from the oviduct, and an ultrafine glass pipet is used to inject a solution containing a few hundred copies of the transgene DNA into the nucleus. The injected eggs are put into the oviduct of a surrogate mother, and pups are born 19 days later. Some of these mice will have the transgene in their genome, and their descendants for generations to follow will also carry the transgene. A mouse receiving the transgene is referred to as a transgenic founder; its descendants are members of a genetically identical transgenic line.

The methods to make transgenic mice were developed in the early 1980s by John Gordon and by Ralph Brinster and Richard Palmiter and their co-workers. The first use of transgenic mice was to study gene function in the whole animal; in particular, how and why a specific gene is "turned on" in some tissues and "turned off" in others. Although all of the cells in the body contain an identical set of genes, some genes are active in only one or a few tissues. It is this diversity of gene expression that produces the distinct cell types and tissues of the body, making a muscle cell different from a liver cell.

The two main parts of a gene are the regulatory region and the protein-coding region. When the right combination of proteins binds to specific sites along the DNA in the regulatory region, the gene is switched on, and the protein-coding region becomes active. Transgenic mice have been used to identify the exact parts of the regulatory region that turn a gene on in a particular tissue. To do this, a reporter gene is made by fusing different parts of the regulatory region of one gene onto the protein-coding region of another gene. The protein-coding region is usually taken from a gene not found in the mouse, often the β-galactosidase gene of bacteria. The β-galactosidase gene codes for an enzyme that produces a blue color when exposed to a specific chromogenic substrate called X-Gal. Only those mice that receive the part of the regulatory region needed to activate the gene will develop the blue color in the appropriate tissue.

The knowledge gained about the regulation of expression of specific genes using transgenic mice is likely to have important implications for future environmental health research. For example, the level of activity of a gene that codes for a detoxifying enzyme may determine a person's susceptibility to a toxic environmental agent. This hypothesis could be tested in different lines of transgenic mice designed to express the gene at high or low levels. Such mice might also be used to detect when exposure occurs, define the mechanisms leading to the toxic effects, and develop treatments for individuals exposed to the agent.

Transgenic mice have also proven to be valuable for understanding the roles of proteins. Using transgenic mice, it is possible to increase or decrease the amount of specific proteins, express proteins at a different time or in a different tissue than normal, and test the function of a modified protein. For example, one of the first transgenic mice lines had a transgene with the regulatory region of the mouse metallothionein gene spliced to the coding region of the rat growth-hormone gene. These mice grew to be twice as large as normal mice. This study dramatically demonstrated that the protein coded by the transgene was functional in the mouse and that rat growth hormone produced an additive effect on mouse growth hormone.

Transgenic mice offer new ways to identify and characterize environmental, occupational, genetic, dietary, and other factors that induce neoplastic processes. Two classes of genes have been identified that influence tumor formation: Tumor-suppressor genes act in a negative manner to control cell growth, and oncogenes appear to function in a positive fashion. One gene being studied is the p53 tumor-suppressor gene, whose product appears to be involved in maintaining genomic stability. Mutations that inactivate the p53 gene are the most common genetic alteration observed in a wide variety of human cancers. Compared to normal animals, mice that carry a mutant p53 transgene are much more susceptible to lung tumor formation after treatment with a chemical carcinogen. Tumors from these mice are being examined to identify additional tumor-suppressor genes that interact with the transgene. These studies may identify novel genes involved in cancer development and help determine the relationship between genetic diversity and variable susceptibility to cancer.

The protein-coding region of oncogenes has been introduced into mice with regulatory sequences that target certain tissues and allow activation of the transgene by a specific chemical or genetic mutation. For example, transgenic mice carrying an activated oncogene that is sensitive to carcinogenic and promoter effects of

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Mighty mouse. Transgenic mice are powerful tools for solving human gene puzzles.

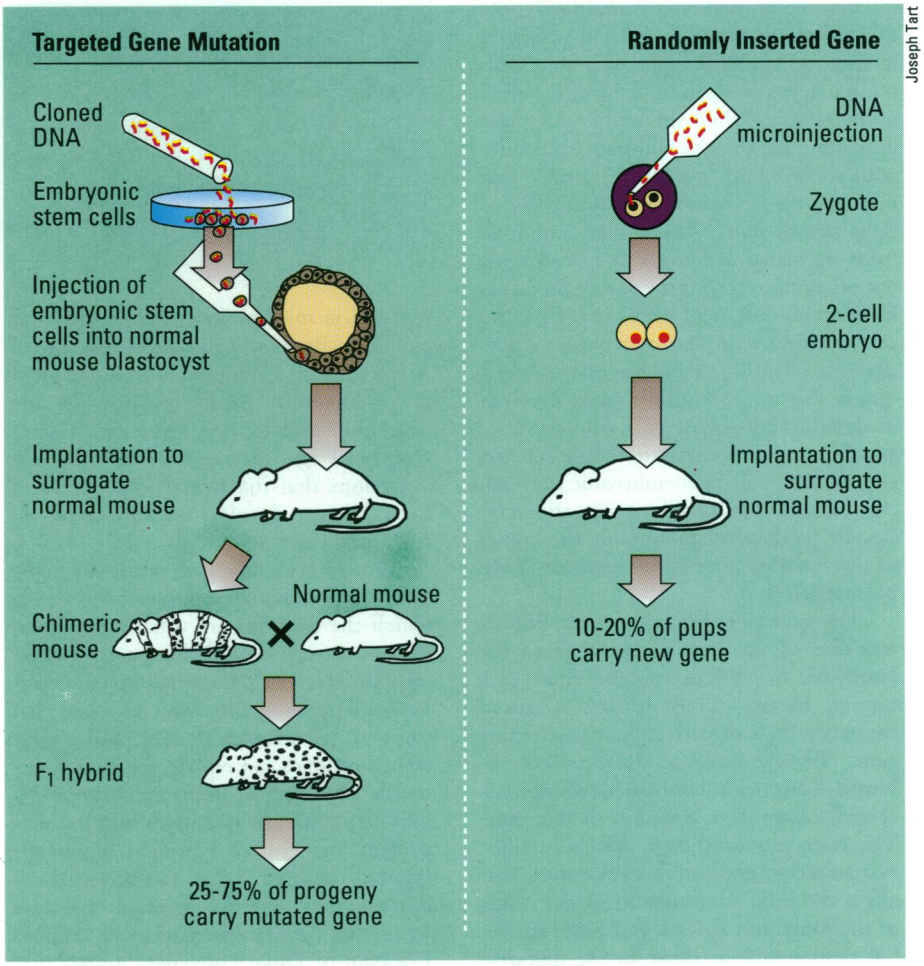
environmental chemicals applied to the skin are being studied. These mice respond to certain chemicals with a different pattern of papillomas or carcinomas than animals lacking the transgene.

Such transgenic mice provide a sensitive way to study the rate and pattern of tumor formation, the range of tissues affected, and the interaction of different factors that influence tumor formation. Researchers hope to make some transgenic mice more sensitive to the carcinogenic effects of certain chemicals than other animals to more rapidly detect carcinogens.

Big Blue transgenic mice were developed in a collaborative effort between NIEHS and Stratagene. All of the cells in these mice contain an inactive β-galactosidase reporter transgene. Exposure of the mice to mutagenic chemicals causes mutations that activate the transgene. These mutations are identified by isolating DNA from various transgenic mouse tissues, transferring it to bacterial hosts, and scoring the activity of β-galactosidase *in vitro*. This approach is a relatively rapid and efficient way to screen *in vivo* for chemical mutagens. In addition, mouse DNA can be extracted from the bacterial colonies, and the specific nature of the mutation can be determined from the nucleotide sequence.

Transgenic mice have also been used to identify previously unknown genes. Transgenes are incorporated randomly into the genome of the fertilized egg and occasionally disrupt a gene by being inserted into its regulatory or protein-coding region. The result is mice with genetic mutations such as limb deformities, infertility, reversal of left-right asymmetry, or lethal developmental anomalies. However, the transgene serves as a marker for the site of mutation and can be used to clone the disrupted gene. This allows identification of a candidate gene for comparable birth defects in humans. Such findings have led to unique approaches for identifying genes involved in development. In "gene trapping," transgenes that lack regulatory sequences but that contain the bacterial β-galactosidase coding region are injected. These transgenes are expressed when they are incorporated into a location in the genome that allows them to be activated by the regulatory portion of a gene. The X-Gal labeling approach can then be used to define the temporal and spatial expression of the trapped gene, and the transgene can be used as a marker for cloning that gene.

The NIEHS, National Academy of Sciences Institute of Laboratory Animal Resources (ILAR), and Oak Ridge National Laboratory Human Genome Group have combined efforts to establish a database that catalogs information on transgenic animals. Researchers use standardized nomenclature to submit information about their transgenic animal lines. The computerized database includes details of the transgenic constructs, expression patterns of transgenes, the morphological and functional effects of the transgenes, and other information about the genetics and availability of the animals. [For additional information about the transgenic animal database, contact Karin Schneider at Oak Ridge National Laboratories, (615) 574-6529.]



How to build a better mouse. Scientists are using two innovative techniques to create transgenic animals.

Targeted Gene Mutations

It became possible to mutate specific genes in the late 1980s with the convergence of advances in developmental, reproductive, and molecular biology. Targeted mutations have allowed the development of mouse models of human genetic diseases and tests of the roles of specific genes by causing loss of function ("gene knockout"). One key technical advance was the development of methods for growing embryonic stem cells from mouse embryos in tissue culture. When embryonic stem cells are injected into mouse embryos at an early stage of development, they affect all the tissues of the mouse. Embryos are removed on the third day of development, injected with embryonic stem cells, and transferred to surrogate mothers. Some of the mice born are chimeras, composed of a mixture of cells from the original embryo and from embryonic stem cells. The sperm or eggs in the chimeric mouse give rise to offspring carrying the embryonic stem cell genes.

The other key advance in producing mice with genes knocked out was techniques to cause mutations that block the function of specific genes in embryonic stem cells. This first requires cloning the gene and modifying it using genetic engineering methods. The modifications inactivate the normal function of the gene, but add the ability to inactivate a toxic drug. The normal gene in an embryonic stem cell is replaced with the modified gene by a procedure known as homologous recombination. Embryonic stem cells that contain the modified gene are selected by their resistance to the drug and subsequently injected into mouse embryos to make chimeric mice.

Coat-color markers are used to identify the mice carrying the genes from embryonic stem cells. Most embryonic stem cells were originally derived from mice with white coats. When the embryonic stem cells are injected into embryos from mice with a black coat, the chimeric mice that result have a mixture of black and white or light brown hair. When these chimeric mice are mated to mice with a black coat, the offspring receiving a copy of the genes from embryonic stem cells often have tan coats. However, other coat colors are possible for offspring of the original chimeric mouse due to the multiple genes involved in determining mouse coat colors. Since an offspring of a chimeric mouse inherits only one copy of each embryonic stem cell chromosome, additional matings are necessary to produce offspring with two copies of the mutated gene in the absence of the normal gene.

The gene responsible for cystic fibrosis was one of the first to be targeted for knock-out to produce a mouse model of a human disease. Cystic fibrosis is caused primarily by a defective chloride-channel gene. Beverly Koller at the University of North Carolina School of Medicine successfully targeted a mutation to this gene. The mice produced have defects in chloride secretion and sodium absorption that affect the fluid environment of the lining of the lungs and airways and other epithelial tissues such as those in the digestive and reproductive tracts. These mice have been a major advantage in studying cystic fibrosis. The mice are being used to better understand how mutations in the gene lead to the disease, test different drug treatments, and develop gene therapy strategies.

Inherited mutations in the p53 tumor-suppressor gene give rise to the Li-Fraumeni syndrome in humans, characterized by a profound susceptibility to several forms of cancer. When the p53 gene was mutated in mice by Lawrence Donehower and Allan Bradley at the Baylor College of Medicine, these animals developed normally but were prone to spontaneous development of a variety of neoplasms by 6 months of age. About 75% of mice with mutations that inactivate both copies of the p53 gene develop multiple types of tumors by this age.

One of the surprising results of many gene knock-out studies is that the tissues in which the proteins are usually expressed are not disrupted, and there are no other obvious effects of the mutation. An explanation frequently proposed has been that some proteins are redundant, and closely related proteins can replace the ones eliminated. Other explanations are that the animal can function normally without the protein, that loss of a protein might up-regulate compensatory mechanisms, or that proteins may be expressed in tissues where they have no function at all. Harold Erickson of Duke University argues that protein synthesis is cheap from the perspective of cellular metabolism, but that gene control mechanisms are expensive. He suggests that unless the protein is deleterious to the cell, there is no compelling reason for the cell not to make a superfluous or junk protein. However, the number of examples where gene knock-outs have no obvious effects are small, and it remains to be seen if there are subtle but important effects in these animals.

Gene knock-out technology is still at an early stage of development and is difficult and inefficient. To make it more usable, the availability and stability of embryonic stem cell lines, the efficiency of targeting mutations to specific genes in embryonic stem cells, and the frequency by which chimeras produce offspring that carry the gene knock-out must be improved. The recent development of embryonic germ cell lines from primordial germ cells may reduce some of these technical problems. In addition, gene knock-outs are often made by removing part of a gene and inserting a piece of foreign DNA in its place, using a drug-resistance gene isolated from bacteria. Continuing improvements in technology are allowing more subtle changes to be introduced into genes, such as producing specific deletions or substitutions in the regulatory or coding regions that mimic human disease mutations without permanently inserting foreign DNA. As more genes are identified from experimental studies and the human genome project, transgenic mice will provide more powerful ways to test how genes interact with the environment to affect human health.

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Suggested Reading

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